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Dispersal in a patchy landscape reveals contrasting determinants of infection in a wild avian malaria system

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Running head: Timing and location of avian malaria infection

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Summary

1. Understanding exactly when, where and how hosts become infected with parasites in the wild is critical to understanding host-parasite coevolution. However, for host-parasite systems where hosts or parasites are mobile (e.g. vector-borne diseases), the spatial location of infection, and the relative importance of parasite exposure at successive host life-history stages, are often uncertain.

2. Here, using a six-year longitudinal dataset from a spatially referenced population of blue tits, we test the extent to which infection by avian malaria parasites is determined by conditions experienced at natal or breeding sites, as well as by postnatal dispersal between the two.

3. We show that the location and timing of infection differs markedly between two sympatric malaria parasite species. For one species (*P. circumflexum*), our analyses indicate that infection occurs after birds have settled on breeding territories, and because the distribution of this parasite is temporally stable, hosts could in principle alter their exposure and potentially avoid infection through postnatal dispersal. Conversely, the spatial distribution of another parasite species (*P. relictum*) is unpredictable, and infection probability is positively associated with postnatal dispersal distance, potentially indicating that infection occurs during this major dispersal event.

4. These findings suggest hosts in this population may be subject to divergent selection pressures from these two parasites, potentially acting at different life-history stages. Because this implies parasite species-specific predictions for many coevolutionary processes, they also illustrate the complexity of predicting such processes in multi-parasite systems.

Introduction

In natural populations, individuals vary markedly in their exposure and susceptibility to parasitic infections (Combes 2001). As with any phenotype, variation in infection-related traits will be underpinned by a combination of genetic, environmental and parental effects (Mackinnon et al. 2005, Mitchell and Read 2005, Wolinska and King 2009), acting at various stages throughout an individual's lifetime. Elucidating the relative contributions of these factors, and the life-history stages at which they operate, constitutes a key challenge in any host-parasite system, and is fundamental to understanding how host physiology, behaviour and life-histories evolve in response to parasitism.

Understanding when and where wild hosts become infected can be difficult, particularly when exposure is not known to be confined to a particular life-history stage, and hosts are not sessile. Since abiotic factors, such as climate and habitat, are known to affect the distribution of parasites and their vectors, spatial variation in infection prevalence is often assumed to reflect environmental variation in parasite exposure across sampling locations. However, if hosts are mobile, and particularly when infections are long-lasting, hosts may acquire infection at a range of locations over their life-histories, and host movements, as well as vector movements for vector-borne diseases, can therefore play a role in driving spatial variation in prevalence (Bodker et al. 2006, Farnsworth et al. 2006). Host movements may be particularly important at small spatial scales, and could significantly influence within-population spatial variation in infection probability. For example, infection may reduce hosts' ability to disperse (Heeb et al. 1999, Fellous et al. 2011) or to compete for and settle

on high quality territories, leading to clustering of infected hosts or increased infection prevalence in marginal habitats.

Although challenging, elucidating the spatiotemporal and life-history context of infection is critical for understanding host-parasite coevolutionary processes. For instance, how hosts encounter parasites in time and space is expected to affect the evolution of parasite virulence and host resistance (Boots and Mealor 2007, Wild et al. 2009, Best et al. 2011) and the potential for host-parasite local adaptation (Kawecki and Ebert 2004, Foster et al. 2007, Gandon and Nuismer 2009). Furthermore, the life-history stage at which hosts encounter parasites will influence the type of defence mechanisms hosts evolve. For instance, if infections are generally acquired early in life and offspring are reliably exposed to similar parasites as those affecting their parents, selection may favour the evolution of adaptive maternal effects such as maternal antibody transfer (Gasparini et al. 2001, Grindstaff et al. 2003, Sadd and Schmid-Hempel 2009). Conversely, for infections acquired later in life, short-lived maternal effects such as antibody transfer are unlikely to be beneficial. In such cases, behavioural mechanisms of parasite avoidance including biased dispersal, settlement or mate choice (Boulinier et al. 2001), or host resistance or tolerance (Little et al. 2010) may be selected for.

Avian malaria parasites (*Plasmodium* spp.) are a globally widespread group of vector-borne parasites, used as a model system for investigating many aspects of host-parasite interaction and coevolution (Ricklefs and Fallon 2004, Hellgren et al. 2009, Bensch et al. 2009, Marzal et al. 2011). Spatial variation in the prevalence of these parasites has been widely documented, at broad geographic scales as well as

within host populations (Bensch and Åkesson 2003, Wood et al. 2007, Sehgal et al. 2011). However, since birds are highly mobile, haemosporidian infections are chronic (Valkiūnas 2005), and vector ecology is often not well understood, the exact timing and location of infection within hosts' life-history is often unknown. Moreover, the majority of existing studies on these parasites are cross-sectional, with individuals or populations sampled only once. Longitudinal studies, in which individuals are captured multiple times throughout life are rare, yet essential for understanding how events at different life-history stages determine infection. Here we use data from a long-term study of a wild, spatially referenced population of blue tits (*Cyanistes caeruleus*) affected by malaria parasites to ascertain how exposure at different life-history stages determines infection, and the potential role of host dispersal in this process. Previous work on this population has demonstrated consistent spatial variation in infection probability according to breeding location for one of two common malaria parasite species (*Plasmodium circumflexum*), while there is little spatial structure in prevalence for the other species (*P. relictum*) (Wood et al. 2007, Knowles et al. 2011, Lachish et al. 2013). Despite such contrasting spatial patterns, when and where infection occurs for each species, and the relative importance of conditions experienced early in life vs. later in life as adults, remains unclear. Blue tits are territorial during breeding, and the major dispersal event occurs between fledging the nest and settling to breed, with movements between successive breeding attempts generally being far more limited (Matthysen et al. 2005; see below for data from this study). Thus several transmission scenarios could drive spatial variation in *P. circumflexum* infection probability across breeding sites, including: (1) spatial variation in exposure to parasites at breeding sites (2) spatial variation in exposure across natal sites followed by limited postnatal dispersal (3) infection of hosts before postnatal dispersal (with or without spatial

variation in infection risk) followed by non-random settlement of infected and non-infected birds. Similarly, whilst *P. relictum* prevalence is not spatially predictable across breeding sites (Lachish et al 2013), several possible scenarios of host exposure could underlie this observation, including: (1) spatial variation in exposure at natal sites, followed by disruption of this spatial pattern by subsequent postnatal dispersal (2) infection occurring during postnatal dispersal (3) infection occurring at breeding sites, with the scale of environmental variation in parasite exposure smaller than that examined to date, (4) no spatial variation in exposure on the scale examined, perhaps because vectors fly long distances between acquiring and transmitting infection among hosts. In the population studied here, the vector species responsible for avian malaria transmission remain uncertain. In the absence of knowledge about vector ecology that could be used to predict malaria exposure risk at different life-history stages (e.g. vector spatial distribution, competence, biting rate and dispersal), we took an alternative approach, using host infection data alone to infer the location and timing of infection. Using six years' of data on locally hatched birds that later bred in this population, we test whether infection probability as a breeding adult is best predicted by local parasite prevalence among the breeding population surrounding their natal or breeding sites, and how dispersal distance may modulate these effects. Assuming that birds acquire infection from a reservoir of infected hosts in their local vicinity via a mosquito bite, and that these prevalence measures are therefore a proxy for exposure risk at that time and place, this should test whether infection more often occurs while they are at natal or breeding sites. We also test for parent-offspring associations in infection status, to examine whether a shared environment between parents and offspring can drive similarity in their infection status, and whether this is modulated by dispersal distance. Most strikingly, our

results suggest key differences in how and when hosts come to be infected by these two sympatric *Plasmodium* species, suggesting they may exert divergent selection pressures within this single host population.

Materials and Methods

Field procedures

The study was conducted in 2004-9 in Wytham Woods (51˚46'N, 1˚20'W), a 385ha mixed deciduous woodland near Oxford, UK, where approximately 250-450 blue tit pairs breed annually in nestboxes. During the breeding season each year (April-June), all (c.1160) nestboxes at the study site were monitored at regular intervals, such that hatch date (date on which the first egg hatched) was determined for all reproductive attempts. Where possible, all parents were captured when their brood was between day 6 and 15 post-hatch, either within the nestbox by hand or using traps, or with mist nets in front of the nestbox entrance, and blood samples were taken under licence by brachial or jugular venepuncture. Thus all birds were sampled at a standardised point in their annual cycle. Adults were sexed based on the presence (female) or absence (male) of a brood patch and age determined by plumage characteristics. All nestbox-born nestlings were ringed such that their recruitment to the breeding population could be monitored.

Diagnosing malaria infections in recruits and their parents

Genomic DNA was extracted from all blood samples. For samples from 2004-2005, DNA was extracted using DNeasy extraction kits (Qiagen), whilst in 2006-9 a standard ammonium acetate method was used. Two divergent clades of *Plasmodium* parasite are commonly detected in this population (based on cytochrome *b* sequences

obtained by nested PCR), which correspond to two well-defined morphospecies, *P. relictum* and *P. circumflexum* (Palinauskas et al. 2007, Knowles et al. 2011). For simplicity we refer to them by their morphospecies classification from here on. We used a quantitative (q)PCR assay for detecting and quantifying *Plasmodium* parasites, and determining the infecting species; *P. relictum* and *P. circumflexum* can be reliably diagnosed according to the melting temperature of their qPCR products, which differ by approximately 1˚C. qPCR reactions were performed exactly as described in Knowles *et al.* (2011), with parasitaemia scored as the average number of *Plasmodium* DNA copies across three replicate wells (including zero counts), and a sample diagnosed as positive if any of the three replicate wells contained a parasite-specific product. All samples from 2005-9 were screened using this qPCR assay. Samples from 2004-7 were additionally screened using a nested cyt *b* PCR (Waldenström et al. 2004). Thus for (parental) samples taken in 2004, only nested PCR diagnosis was available, and for 2008-9 (recruit and parental) samples only qPCR diagnosis was available. Samples taken between 2005-7 were screened by both methods. These two methods show a high level of agreement (Knowles et al. 2011), however to maximise diagnostic accuracy we used a consensus measure of malaria infection where diagnoses from both methods were available: if one or both assays gave a positive diagnosis an individual was recorded as infected. Since multiple negative controls were used in all PCRs and plates were repeated in the rare cases where any of these tested positive, false positives are very unlikely. Application of occupancy modelling in this system also suggests the rate of false negatives is low (Lachish et al. 2012).

Characterising natal and breeding sites

Defining natal and breeding territories

The exact locations (x and y coordinates) of all nestboxes in Wytham are known with some precision as these were digitally mapped to an accuracy of ±3m using differential GPS in 2004-5 (Wilkin et al. 2006). Since tits are confined to the nestbox for the first few weeks of life, and as breeding adults are territorial and forage in the immediate vicinity of the nest (Naef Daenzer et al. 2000, Stauss et al. 2005), these coordinates give an accurate description of an individual's location both during their own nestling phase and as a breeding adult. To estimate an individual's territory during its natal and breeding years, tessellated polygons (Thiessen polygons) were formed by placing boundary lines equidistant between occupied nestboxes in each breeding season, using the GIS software MapInfo Professional v7.8. The area of these polygons provides an individual-specific measure of territory area (Wilkin et al. 2007). Since great tit territories are interspersed with blue tits in this population, and interspecific competition occurs between the two species (Minot and Perrins 1986) we included great tit territories (inferred from parallel studies of this species at this site) when calculating the area of Thiessen polygons for blue tits.

Deriving measures of malaria exposure risk at natal and breeding sites

We used the interpolated local prevalence of malaria among breeding adult blue tits surrounding each territory as a measure of malaria exposure risk at that specific place and time. To ascertain where and when infection occurred, we tested whether infection status of recruits for a given *Plasmodium* species was best predicted by these local prevalence estimates at their natal or breeding territories in the appropriate year. Figure 1 provides a schematic overview the analytical approach, which is

described in detail below. GIS software (MapInfo Professional v7.8) was used to create interpolated maps of malaria prevalence among breeding adults in each of the breeding seasons 2004-2009, using consensus infection data from qPCR and nested PCR, as described in Wood *et al.* (2007). By overlaying territory boundaries (Thiessen polygons) on these prevalence interpolations, estimated local prevalence within each territory in each year was then extracted, for each of the two *Plasmodium* species separately. Throughout this paper, the term 'local prevalence' is used to refer to these measures of territory-level interpolated prevalence. When testing whether local prevalence at breeding sites predicts recruit infection status, the focal individual's infection status should not be included when generating interpolated prevalence, as this would entail some circularity. To achieve this, interpolations of prevalence at the breeding territory were always derived excluding infection data from all recruits in the dataset. This potential problem of circularity does not apply to measures of natal territory malaria risk, as all recruits were nestlings in their year of birth, and therefore not sampled and unable to contribute to prevalence interpolations. However, the exclusion of recruits will influence the resolution of malaria interpolations (each year 17-29% of individuals were recruits). Thus, in analyses that compared the explanatory power of local prevalence estimates at natal and breeding sites, both measures were derived using infection data that excluded recruits, to ensure these measures were of comparable resolution. For the same reason, recruits hatched in 2004 were excluded from these analyses, as the resolution of the breeding and natal interpolations were different (breeding interpolation excluded recruits, but natal interpolation was complete as no individuals in this dataset were breeding adults in 2004). In analyses considering local prevalence at the natal site only, interpolations using all breeding adults were used, to maximise statistical power.

Measuring postnatal dispersal

Postnatal dispersal distance was defined as the Euclidean distance between the nestbox where an individual was raised as a nestling, and the nestbox in which it was first captured breeding. Since dispersal distances between breeding attempts are generally much shorter than postnatal dispersal distances in blue tits (dispersal distance median and IQR in this dataset: postnatal 678m (325-1246m) n=447; breeding 48m (0-88m) n=111), postnatal dispersal reflects the major dispersal event in this system.

Dataset and statistical analyses

A total of 447 recruits hatched in 2004-2008 and caught as adults between 2005-2009 were included in analyses. The majority (78%) were captured breeding for the first time as yearlings, the remainder being captured for the first time as 2 year-olds (18%) or older (4%). Only the first breeding capture for each recruit was included in analyses to avoid pseudoreplication, and analyses were performed for the two parasite species, *P. circumflexum* and *P. relictum*, separately. All analyses were performed in R version 2.13.2 (R Development Core Team, 2011). Since some broods contributed multiple recruits to the dataset (mean=1.3 recruits per brood), where recruit infection status or parasitaemia was the response variable, brood was entered as a random effect. However, since the majority of broods (76%) contributed only one recruit, GLMs were also performed and gave extremely similar results in all cases (results not shown). Unless otherwise stated, model selection involved backwards stepwise simplification of full models using likelihood ratio tests to sequentially remove terms $p>0.05$, leaving the minimum adequate model.

Generalized linear mixed models (GLMMs) with a binomial error structure were used to test whether recruit infection status was predicted by local prevalence at natal or breeding sites in univariate analyses, controlling for natal year. Since estimates of local prevalence at individuals' natal and breeding sites were not strongly collinear (see Results), we then included both terms in a model (again with natal year as a covariate), to test which more strongly predicted recruit infection status. Under the assumption that local adult birds act as the source of infection for recruits, this analysis can test whether infection more likely occurs during the nestling/fledging or breeding period.

If infection occurs after postnatal dispersal, and if infection risk is spatiotemporally stable, the extent to which natal site local prevalence predicts infection status may depend on postnatal dispersal distance. To test this possibility, we fitted models predicting recruit infection status that included interactions between natal site prevalence and postnatal dispersal distance, including natal year as a covariate.

These models predicting infection status from local prevalence at natal and breeding sites were also run with parasitaemia (log-transformed *Plasmodium* DNA copies among infected individuals) as the response variable, to assess whether any patterns seen with infection probability could be driven by parasitaemia-related differences in infection detectability.

Two additional analyses were performed to place our results in context. To assess the spatiotemporal consistency in prevalence for each *Plasmodium* species, we examined

the correlation in estimated local prevalence at territories occupied in consecutive years, using only the first pair of years where a nestbox was inhabited by different yearling birds to ensure a positive correlation could not be driven by individuals breeding repeatedly on the same territory. Second, we examined the level of spatial autocorrelation in prevalence across breeding sites for both *Plasmodium* species, using both omnidirectional and directional semivariograms constructed in the R package geoR. A nestbox was deemed positive if at least one of the current breeding pair was infected. For each semivariogram, a simulation envelope was constructed from 999 Monte Carlo permutations of the data, to assess whether the level of spatial autocorrelation was above that which could occur by chance.

Binomial GLMMs were used to test whether recruit infection status was predicted by infection status of either parent or the total number of parents infected (where the status of both parents was known), as diagnosed in the recruit's year of birth. A positive association in infection status between parents and offspring could occur if they are exposed at the same place and time (e.g. during the recruit's nestling period/parents breeding period), or if they are both exposed as breeding adults, but offspring do not disperse far from their natal sites. The latter scenario would predict any positive parent-offspring association to diminish with increasing postnatal dispersal distance. We tested for this by assessing the significance of interaction terms between parental infection variables and postnatal dispersal distance. Other biological processes could also influence parent-offspring associations in infection status. Transfer of protective antibodies from mothers to offspring would push the association between maternal (but not paternal) infection and offspring infection in a negative direction, whereas heritability of malaria susceptibility would push the

association in a positive direction. With only antibody transfer or genetic effects, no dependence of the association on recruit postnatal dispersal distance is expected. Natal year and recruit age (as a covariate) were controlled for in these models. We also examined the association between parental infection status and offspring parasitaemia, both considering all *Plasmodium* infections and cases where parent and offspring were infected by the same *Plasmodium* species, whilst controlling for sampling year, which is known to influence parasitaemia (Knowles et al. 2011). 84 recruits (19%) were not raised in their natal nest due to cross-fostering or brood size manipulation experiments during the course of the study. The data for these recruits was insufficient to effectively compare the relative influence of natal and rearing parental infection status, and therefore they were excluded from analyses of parental effects.

Results

Timing and location of P. circumflexum infection

P. circumflexum infection probability was positively predicted by the local prevalence of this parasite at both recruits' natal and breeding sites in univariate analyses (Table 1). This is because local prevalence at individuals' natal and breeding territories is positively correlated (r=0.385, 95% CI: 0.291 to 0.472), likely due to a limited extent of postnatal dispersal, as evidenced by strong correlations between recruits' natal and breeding nestbox coordinates (x coordinate: r=0.578, 95% CI: 0.513 to 0.636; y coordinate: r=0.608, 95% CI: 0.546 to 0.663), and the consistent spatial distribution of this parasite across years. In line with previous findings showing consistent spatial clustering of this parasite in the northern part of the site (Knowles et al. 2011, Lachish

et al. 2013), here we find at the level of individual territories, there is strong spatial repeatability in infection risk: prevalence at a given nestbox in one year strongly and positively predicted prevalence at the same site the following year (Pearson's r=0.72, $F_{1,155}$= 169.74, p<0.001). When local prevalence of *P. circumflexum* at both natal and breeding sites were entered in the same model predicting recruit infection status, only breeding site prevalence remained significant (Table 1). Moreover, the ability of natal site prevalence to predict recruit infection was clearly dependent on postnatal dispersal distance (Table 2). When dispersal distance was short, natal site prevalence strongly and positively predicted infection probability, but this effect diminished substantially with increasing postnatal dispersal (Fig. 2a). Together, these results indicate that *P. circumflexum* infections are likely acquired after postnatal dispersal, once birds have settled on their breeding territories. Since postnatal dispersal distance is strongly associated with sex in this population (Median and IQR for dispersal distance: Females: 952m, 499-1568m, n=214; Males: 531m, 258-983m, n=323; Wilcoxon signed-rank test Z=5.90, p<0.001), we tested whether the effects reported above were either driven by sex differences or were sex-dependent. A significant 3-way interaction suggested dispersal modulated the influence of natal prevalence on *P. circumflexum* infection probability more strongly for males than females (Natal prevalence*Dispersal*Sex χ^2_1=3.84, p=0.050). To explore the spatial scale over which dispersal could alter infection probability, we plotted semivariograms to examine spatial autocorrelation in prevalence across breeding sites. Although an omnidirectional semivariogram provided limited evidence of spatial autocorrelation for *P. circumflexum* (Fig. S1), directional semivariograms indicated positive spatial autocorrelation along the east-west axis (0 degrees), whereas autocorrelation in a north-south direction (90 degrees) was negative (Fig. S2). Thus postnatal dispersal direction, as well as distance, will likely affect the

extent to which dispersal can alter infection probability. Since the sexes may differ in breeding site settlement behaviour, we tested whether local prevalence at breeding sites predicted infection differentially for the two sexes, and found that breeding site prevalence predicted *P. circumflexum* infection status more strongly for males than females (Sex*Breeding site prevalence interaction: χ^2_1=12.18, p<0.001, n=442 Fig. S3). Neither local breeding nor natal prevalence strongly predicted *P. circumflexum* parasitaemia among infected individuals, suggesting parasitaemia differences do not drive the patterns reported here (Table S1). The likelihood that recruiting blue tits were infected by *P. circumflexum* was positively predicted by how many of their parents were infected with this parasite, paternal infection status alone, and to a lesser extent by maternal infection status (Table 3). However, these positive parent-offspring associations were also affected by postnatal dispersal behaviour. Infection with *P. circumflexum* was more strongly predicted by the number of infected parents when postnatal dispersal distance was short (Fig. 3; Number parents infected*dispersal distance χ^2_1=4.15, p=0.042, n=266), indicating that this association is at least partly driven by a common level of exposure to infection among parents and those offspring that dispersed short distances from their natal site. However, in models where recruit exposure was controlled for, by inclusion of *P. circumflexum* local prevalence at their breeding site as a model term (instead of a dispersal interaction term), the number of parents infected still significantly predicted recruit infection status (Number of parents infected: χ^2_1= 9.57, p=0.002, n=266). Thus, there may be a positive association between parental and offspring *P. circumflexum* infection status over and above that generated by similar exposure. The effect of individual parent infection status' remained positive but had less predictive power in these models (Maternal status: χ^2_1= 1.95, p=0.162, n=305; Paternal status: χ^2_1= 4.01, p=0.045, n=309).

Timing and location of P. relictum infection

For *P. relictum*, recruit infection status was not predicted by local prevalence at either natal or breeding sites, in either univariate analyses or when both terms were entered in the same model (Table 1). There was no correlation in estimated local prevalence across individuals' natal and breeding territories for this parasite (r=-0.040, 95% CI: -0.145 to 0.066), which is likely because even though dispersal is limited, this parasite showed little consistency in its spatial distribution across years: even at the same nestbox, local *P. relictum* prevalence in one year did not predict prevalence at the same site the following year (Pearson's r=-0.07, $F_{1,155}$=0.73, p=0.395). This is in line with previous findings from this population showing little similarity in the broad-scale spatial distribution of this parasite across years (Lachish et al. 2013). There was no significant interaction between natal site prevalence and postnatal dispersal distance for *P. relictum* (Table 2), but a main effect of dispersal distance was found, indicating that individuals that had dispersed further from their natal site had a higher probability of *P. relictum* infection (Table 2, Fig. 2b). In analyses considering the potential role of sex in these effects, there was some evidence that the effect of natal site prevalence differed among the sexes, being slightly negative among females and slightly positive among males (Natal prevalence*Sex: χ^2_1=4.54, p=0.033), but no evidence that the main effect of postnatal dispersal distance was sex-dependent (Dispersal*Sex: χ^2_1=0.38, p=0.540) or of a 3-way interaction (Natal prevalence*Dispersal*Sex: χ^2_1=0.03, p=0.858). There was no evidence for spatial autocorrelation in *P. relictum* infection probability across breeding sites, considering both omnidirectional (Fig. S1) and directional semivariograms. There also was no evidence that sex altered the predictive ability of breeding site prevalence for *P. relictum* (Sex*Breeding site prevalence

interaction: *P. relictum* χ^2_1=0.22, p=0.643, n=442). As with *P. circumflexum, P. relictum* parasitaemia was not predicted by either local breeding or natal prevalence of this parasite (Table S1). No significant associations between parental and offspring infection status were found for *P. relictum* (Table 3), and the interaction between the number of infected parents and dispersal distance was not significant (*P. relictum* χ^2_1=0.01, p=0.903, n=266).

Maternal infection status for pooled *Plasmodium* showed no association with offspring parasitaemia (χ^2_1=2.36, p=0.124, n=109). Similarly, when the two parasite species were considered separately, no associations between maternal infection status and offspring parasitaemia were found (*P. circumflexum*; χ^2_1=1.01, p=0.315, *P. relictum* χ^2_1=2.83, p=0.092, n=54).

Discussion

Using longitudinal data on wild birds and their dispersal movements, we examined the life-history stage and location at which infection by avian malaria parasites most likely occurs in a wild blue tit population. Most significantly, we show that the point at which infection occurs during the host's life-history seems to differ markedly for two sympatric *Plasmodium* species, leading to contrasting predictions about how these two parasites may interact with, and impose selection on their hosts. Our results also suggest that an important determinant of infection with these parasites is the pattern and extent of dispersal, presumably driven by how this affects exposure to infective vectors.

Timing and location of malaria infection

In temperate regions, the timing of avian malaria transmission is not well understood, and will depend critically on the specific ecology of the vectors responsible. Data on the seasonal dynamics of avian malaria prevalence in European birds and mosquitoes suggest transmission takes place some time between spring and autumn (Beaudoin *et al.*, 1971; Cosgrove *et al.*, 2008; Ferraguti et al. 2013; Inci et al. 2012). However, for non-migratory passerines, this encompasses several different host life-history stages, including the nestling phase, periods of fledging, dispersal, and settlement on breeding territories. Transmission timing and location will depend on the spatial distribution of vectors, their seasonal dynamics, dispersal, lifespan, biting habits, and vector competence. Moreover, as *Plasmodium* species vary in the vector species they use, their transmission timing will also likely vary. Knowledge about the mosquitoes responsible for transmission, and therefore on these crucial vector ecological parameters, is often lacking for wild avian malaria systems, and can be difficult to obtain. Thus, although predicting the transmission period from vector ecology parameters is possible in theory, in practice there is often insufficient information to do so. In the blue tit population studied here, a diverse assemblage of mosquitoes is present in the woodland (Alves, 2012), but the species responsible for transmitting malaria remain uncertain. Spatial variation in *P. circumflexum* prevalence shows some association with the abundance of one particular mosquito species, however no mosquito species associate with *P. relictum* prevalence (Alves, 2012). Hence, we adopted an alternative approach, using longitudinal data on host movements and infection to infer the likely location and timing of transmission. This suggested divergent transmission biology for two sympatric *Plasmodium* species.

P. circumflexum infection was strongly predicted by local prevalence at recruits' breeding sites, and while prevalence at natal sites did positively predict infection, this was highly contingent on postnatal dispersal distance: natal site prevalence predicted infection among recruits that dispersed short distances before settling to breed, but had no predictive power once postnatal dispersal exceeded 2km (Fig. 2a). These results strongly suggest infection with *P. circumflexum* occurs after birds have settled on, or are at least near to, their breeding territories. This is in agreement with previous findings from this population, including the observation of an autumn peak in *P. circumflexum* prevalence thought to reflect novel transmission (Cosgrove *et al.*, 2008), the inability to detect *Plasmodium* infection in blood samples from 14-day old nestlings (Cosgrove *et al.*, 2006), and data showing total mosquito abundance peaks in mid-July after fledging (Alves, 2012). The finding that breeding site prevalence predicted infection more strongly for males than females could indicate that males arrive at breeding territories earlier, or spend more time close to their eventual territory during *P. circumflexum* transmission. Offspring infection status was positively predicted by how many of their parents were infected, but this positive association declined with increasing postnatal dispersal distance (Fig. 2a). This suggests that a shared environment between parents and offspring can drive similarity in their infection status, and that offspring can potentially escape the fate of their parents by dispersing. However, this positive association remained significant when variation in offspring *P. circumflexum* exposure (local prevalence at their breeding site) was statistically controlled for. This may indicate that genetic similarity in susceptibility is also present, as has been found in bird-ectoparasite systems (Boulinier et al. 1997). Indeed, recent analyses of associations between MHC supertypes and malaria infection in great tits in the same study site (Sepil et al. 2013) suggest the possibility of genetic effects on

malaria infection in tits, as has been found for other passerine birds (Bonneaud et al. 2006, Loiseau et al. 2011, Westerdahl et al. 2012).

In contrast to *P. circumflexum*, *P. relictum* infection probability was not predicted by local prevalence at either natal or breeding sites. Instead, infection probability increased with postnatal dispersal distance. We propose two non-mutually exclusive hypotheses for this parasite's lack of spatial predictability, which we term the 'hosts move' and 'vectors move' hypotheses. First, under the 'hosts move' scenario, transmission of this species may occur at least partly during postnatal dispersal. If hosts move throughout the landscape acquiring infection, this could explain both why neither natal nor breeding site local prevalence predicts infection status, but also the positive effect of dispersal distance - the further an individual disperses, the more likely it is to pass through a patch where *P. relictum* is being transmitted and acquire infection. Our previous finding that *P. relictum* prevalence shows little seasonal variation, and no strong autumn peak in prevalence suggestive of a well-defined transmission season as seen for *P. circumflexum* (Cosgrove et al. 2008), also indicates that infection with this parasite may occur over a longer time-period than for *P. circumflexum*. A longer transmission period could arise through use of a single vector species with a longer biting season, or infection of a broader range of mosquito species with different ecologies. Indeed, the known mosquito host range for morphologically identified *P. relictum* from lab studies is three times higher than for *P. circumflexum* (24 vs. 8 species; Santiago-Alarcon et al. 2012). If *P. relictum* is transmitted by a broader range of mosquitos in Wytham, this could lead to a longer transmission period and reduce the influence of where a bird is at any one time on whether it gets infected or not. A more defined transmission period for *P. circumflexum* than *P. relictum* would also be

predicted from another difference in their seasonal dynamics. While *P. circumflexum* is undetectable in the blood in winter and shows a 'spring relapse' in this population, blood-stage *P. relictum* infections are detectable throughout the year (Cosgrove et al. 2008). The relapse phenomenon is thought to be a parasite adaptation ensuring parasites are only in the blood (and more exposed to host immune responses) when transmission is possible, and thus would be expected more for species with a well-defined transmission season, than those with persistent transmission (Hellgren et al. 2013).

Under an alternative 'vectors move' hypothesis, it is possible that *P. relictum* vectors move long distances between acquisition and transmission of infection. If this were the case, it would violate our initial assumption that local prevalence provides a measure of exposure risk for *P. relictum.* Thus, even if infection occurred at natal or breeding sites, we would not be able to detect it with the analyses performed here.. This 'vectors move' hypothesis cannot explain a positive effect of host dispersal distance on infection probability. However, other factors could drive this, for example if low quality individuals are more susceptible to *P. relictum* infection, and also have to disperse further before securing a breeding territory (de Laet, 1985; Hanski et al., 1991).

Evolutionary implications of species-specific transmission ecology

The ecological differences between *P. circumflexum* and *P. relictum* found here suggest several host-parasite coevolutionary processes will differ for these two species. First, the stage of the host's life-history at which infection occurs plays a key role in determining the type of anti-parasite adaptations hosts may evolve. For example, it will affect the likelihood that dispersal could function as parasite avoidance mechanism

(Boulinier et al. 2001). Several studies of bird-ectoparasite systems have shown that high levels of parasitism may be associated with either increased (Brown and Brown 1992) or decreased (Heeb et al. 1999) emigration or host dispersal distance, and have suggested this may be adaptive and controlled by maternal effects (Tschirren et al. 2007). A common prediction is that dispersal distance should increase with increasing parasitism at the initial location, allowing hosts to avoid parasitism. However, it has also been recognised that spatiotemporal patterns of parasitism will critically influence how dispersal can influence infection probability (Boulinier and Lemel 1996; Boulinier et al. 2001). Our findings on avian malaria clearly demonstrate this, showing that a parasite's spatial distribution and timing of transmission in relation to host dispersal events, determine how postnatal dispersal affects infection probability. For example, in the population studied here, while it is possible that selection could favour a facultative dispersal strategy for avoidance of *P. circumflexum*, with high dispersal (or production of females, the least philopatric sex) in areas of high prevalence and the reverse pattern in areas of low prevalence (Fig. 2a), optimal postnatal dispersal for avoidance of *P. relictum* is much less obvious. Since infection probability increases with dispersal distance, minimal dispersal may minimize infection risk. Overall, the contrasting transmission ecology of these two parasites clearly influences the potential for dispersal to function as a disease avoidance mechanism. This system also illustrates that where multiple parasites with different spatiotemporal patterns occur in the same area, consideration of their relative virulence complicates predictions about optimal dispersal further still.

Species-specific transmission biology will also affect the potential for host-parasite local adaptation. Several studies have examined the evidence for local adaptation in

avian haemosporidia at a genetic level (Bonneaud et al. 2006, Loiseau et al. 2011), yet it is not always clear one should expect it, as the parasites' spatiotemporal ecology is unknown. In Wytham, the same nestboxes repeatedly pose a high risk of *P. circumflexum* infection and since we show that infection occurs after the major dispersal event (postnatal dispersal), there is limited potential for subsequent (much shorter) host movements to transport *P. circumflexum* and break up this environment-driven spatial variation in prevalence. Thus, within-population local adaptation seems possible for *P. circumflexum*. Indeed, recent work has found that fine-scale spatial genetic structure in the Wytham great tit (*Parus major*) population is associated with spatial variation in P*. circumflexum* infection risk (Garroway et al 2013). In contrast, one would not expect local adaptation for *P. relictum,* which shows no consistent spatial distribution, and for which host movements may disrupt any spatial variation in exposure, if they can become infected during postnatal dispersal.

Finally, evolutionary theory suggests the contrasting spatiotemporal ecology of these two parasites may lead to differences in their virulence. A parasite with low dispersal potential and a repeatable spatial distribution like *P. circumflexum* is predicted to have lower virulence than one with a high dispersal ability such as *P. relictum* (Kerr et al. 2006, Boots and Mealor 2007, Wild et al. 2009). Local, rather than global, host-parasite interactions are also predicted to select for greater host resistance (Best et al. 2011). Interestingly, a recent capture-mark-recapture study on this population suggested these two species affect host fitness in different ways, with *P. circumflexum* reducing survival whereas *P. relictum* may reduce the propensity to breed (Lachish et al. 2011), with experimental work also finding an effect of *P. relictum* on breeding performance in blue tits (Knowles et al. 2010). Whether these differences

in fitness effects agree with expectations based on virulence theory remains to be seen, as the relative effect of each species on lifetime reproductive success is not known. However, these parasite species-specific fitness effects and spatiotemporal ecology found within a single woodland, highlight the potential for broader comparative analyses in the diverse group of avian malaria parasites that test the relationship between spatiotemporal ecology and virulence.

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Data Accessibility

References

Alves, R. (2012). Avian malaria associations with British mosquitoes, DPhil Thesis, University of Oxford, Oxford. Beaudoin, R. L., Applegate, J. E., Davis, D.E. & McLean R. G. (1971) A model for the ecology of avian malaria. *Journal of Wildlife Diseases*, 7, 5-13.

Bensch, S., & Åkesson, S. (2003). Temporal and spatial variation of hematozoans in Scandinavian willow warblers. J*ournal of Parasitology* 89, 388–391.

Bensch, S., Hellgren, O. & Perez-Tris, J. (2009) MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial

cytochrome b lineages. *Molecular Ecology Resources* 9, 1353–1358.

Best, A., Webb, S., White, A. & Boots, M. (2011). Host resistance and coevolution in spatially structured populations. *Proceedings of the Royal Society B* 278, 2216–2222.

Bodker, R., Msangeni, H. Kisinza, W. & Lindsay, S. (2006) Relationship between the intensity of exposure to malaria parasites and infection in the Usambara Mountains, Tanzania. *American Journal of Tropical Medicine and Hygiene* 74, 716–723.

Bonneaud, C., Perez-Tris, J. Federici, P., Chastel, O. & Sorci, G. (2006) Major histocompatibility alleles associated with local resistance to malaria in a passerine. *Evolution* 60, 383–389.

Boots, M., & Mealor. M. (2007) Local interactions select for lower pathogen infectivity. *Science* 315, 1284–1286.

Boulinier, T., & Lemel, T-Y. (1996) Spatial and temporal variations of factors affecting breeding habitat quality in colonial birds: some consequences for dispersal and habitat selection. *Acta Oecologica* 17, 531–552

Boulinier, T., Sorci, G. Monnat, J-Y., Danchin, E. (1997) Parent-offspring regression suggests heritable susceptibility to ectoparasites in a natural population of kittiwake *Rissa tridactyla*. *Journal of Evolutionary Biology* 10, 77-85.

Boulinier, T., Mccoy, K. D. & Sorci, G. (2001) Dispersal and parasitism. *Dispersal* (eds J. Clobert, A. Dhondt, E. Danchin & J. Nichols), pp. 169–179. Oxford University Press.

Boulinier, T. & Staszewski, V. (2008) Maternal transfer of antibodies: raising immunoecology issues. *Trends in Ecology & Evolution* 23, 282-288.

Brown, C. R., & Brown, M. B. (1992) Ectoparasitism as a cause of natal dispersal in cliff

swallows. *Ecology* 73, 1718–1723.

Combes, C. (2001) Parasitism: the ecology and evolution of intimate interactions. University Of Chicago Press, Chicago.

Cosgrove, C. L., Knowles, S. C. L., Day, K. P. & Sheldon, B.C. (2006) No evidence for avian malaria infection during the nestling phase in a passerine bird. *Journal of Parasitology* 92, 1302-1304.

Cosgrove, C. L., Wood, M. J., Day, K. P. & Sheldon, B. C. (2008) Seasonal variation in *Plasmodium* prevalence in a population of blue tits, *Cyanistes caeruleus*. *Journal of Animal Ecology* 77, 540–548.

de Laet, J. (1985) Dominance and aggression in juvenile great tits, *Parus major major* L. in relation to dispersal. *Behavioural Ecology: ecological consequences of adaptive behaviour* (eds. R. M. Sibley & R. H. Smith), pp. 375-280. Blackwell Scientific, Oxford.

Farnsworth, M., Hoeting, J., Hobbs, N. & Miller, M. (2006) Linking chronic wasting disease to mule deer movement scales: A hierarchical Bayesian approach. *Ecological Applications* 16, 1026–1036.

Fellous, S., Quillery, E., Duncan, A. B. & Kaltz, O. (2011) Parasitic infection reduces dispersal of ciliate host. *Biology Letters* 7, 327–329.

Ferraguti, M., Martinez-de la Puente, J., Muñoz, J., Roiz, D., Ruiz, S., Soriguer, R., Figuerola, J. (2013) Avian Plasmodium in ulex and Ochlerotatus mosquitoes from Southern Spain: effects of season and host-feeding source on parasite dynamics. PLoS ONE 8, e66237.

Foster, J. T., Woodworth, B. L., Eggert, L. E., Hart, P. J., Palmer, D., Duffy, D. C. & Fleischer, R. C. (2007) Genetic structure and evolved malaria resistance in Hawaiian honeycreepers. *Molecular Ecology* 16, 4738–4746.

Gandon, S., & Nuismer. S. L. (2009) Interactions between genetic drift, gene flow, and selection mosaics drive parasite local adaptation. T*he American Naturalist* 173, 212–224.

Garroway, C.J., Radersma, R., Sepil, I., Santure, A.W., de Cauwer, I., Slate, J. & Sheldon, B.C. (2013) Small scale genetic structure in a wild bird population: the role of limited dispersal and environmentally-based selection as causal factors. *Evolution, in press.*

Gasparini, J., McCoy, K. D., Haussy, C., Tveraa, T., Boulinier, T. (2001) Induced maternal response to the Lyme disease spirochaete *Borrelia burgdorferi sensu lato* in a colonial seabird, the kittiwake *Rissa tridactyla Proceedings of the Royal Society B* 268, 647-650.

Grindstaff, J. L., Brodie, E. D. & Ketterson., E. D. (2003) Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. *Proceedings of the Royal Society B* 270, 2309–2319.

Hanski, I., Peltonen, A. & Kaski, L. (1991) Natal dispersal and social dominance in the common shrew *Sorex Araneus*. *Oikos* 62, 48-58.

Heeb, P., Werner, I., Mateman, A. C., Kölliker, M., Brinkhof, M. W. B, Lessels, C. M. & Richner, H. (1999) Ectoparasite infestation and sex-biased local recruitment of hosts. *Nature* 400, 63–65.

Hellgren, O., Perez-Tris, J. & Bensch, S. (2009) A jack-of-all-trades and still a master of some: prevalence and host range in avian malaria and related blood parasites. *Ecology* 90, 2840–2849.

Hellgren , O., Wood, M. J., Waldenström, J., Hasselquist, D., Ottoson, U., Stervander, M., Bensch, S. (2013) Circannual variation in blood parasitism in a sub-Saharan migrant passerine bird, the garden warlber. *Journal of Evolutionary Biology* 26,

1047-1059.

Inci, A., Yildirim, A., Njabo, K. Y., Duzlu, O, Biskin, Z., Ciloglu, A. (2012) Detection and molecular characterization of avian *Plasmodium* from mosquitoes in central Turkey. *Veterinary Parasitology* 188, 179-184.

Kawecki, T. J., & Ebert, D. (2004) Conceptual issues in local adaptation. *Ecology Letters* 7, 1225–1241.

Kerr, B., Neuhauser, C., Bohannan, B. J. M., & Dean, A. M. (2006) Local migration promotes competitive restraint in a host-pathogen "tragedy of the commons." *Nature* 442, 75–78.

Knowles, S. C. L., Palinauskas, V. & Sheldon, B. C. (2010) Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. *Journal of Evolutionary Biology* 23, 557–569.

Knowles, S. C. L., Wood, M. J., Alves, R., Wilkin, T. A., Bensch, S. & Sheldon, B. C. (2011) Molecular epidemiology of malaria prevalence and parasitaemia in a wild bird population. *Molecular Ecology* 20, 1062–1076.

Knowles, S, Data from "Dispersal in a patchy landscape reveals contrasting determinants of infection in a wild avian malaria system "), Dryad, DOI:, address/URL.

Lachish, S., Knowles, S. C. L., Alves, R., Wood, M. J. & Sheldon, B. C. (2011) Fitness effects of endemic malaria infections in a wild bird population: the importance of ecological structure. *Journal of Animal Ecology* 80, 1196-1206.

Lachish, S., Knowles, S. C. L., Alves, R. Sepil, I., Davies, A. Lee, S., Wood, M. J. & Sheldon, B. C.(2013). Spatial determinants of infection risk in a multi-species avian malaria system. *Ecography* 36, 587-598.

Lachish, S., Gopalaswamy, A. M., Knowles, S. C. L. & Sheldon, B. C. (2012) Site-occupancy modelling as a novel framework for assessing test sensitivity and

estimating wildlife disease prevalence from imperfect diagnostic tests. *Methods in Ecology and Evolution* 3, 339-348.

Little, T. J., Shuker, D. M., Colegrave, N., Day, T & Graham, A. L. (2010) The coevolution of virulence: tolerance in perspective. *PLoS Pathogens* 6, e1001006.

Loiseau, C., Zoorob, R., Robert, A., Chastel, O. Julliard, R. & Sorci, G. (2011) *Plasmodium relictum* infection and MHC diversity in the house sparrow (*Passer domesticus*). *Proceedings of the Royal Society B* 278, 1264–1272.

Mackinnon, M. J., Mwangi, T. W., Snow, R. W., Marsh, K. & Williams, T. N. (2005) Heritability of malaria in Africa. *PLoS Medicine* 2, e340

Marzal, A., Ricklefs, R. E., Valkiūnas, G., Albayrak, T., Arriero, E., Bonneaud, C., Czirják, G. A., Ewen, J., Hellgren, O., Hořáková, D., Iezhova, T. A., Jensen, H., Krizanauskiene, A., Lima, M. R., de Lope, F., Magnussen, E., Martin, L. B., Møller, A. P., Palinauskas, V., Pap, P. L., Perez-Tris, J., Sehgal, R. N. M., Soler, M., Szöllősi, E., Westerdahl, H., Zetindjiev, P. & Bensch, S. (2011) Diversity, loss, and gain of malaria parasites in a globally invasive bird. *PLoS One* 6, e21905.

Matthysen E., Van de Casteele, T. , & Adriaensen, F. (2005) Do sibling tits (*Parus major*, *P. caeruleus*) disperse over similar distances and in similar directions? *Oecologia* 143, 301–307.

Minot, E. O., & Perrins, C. M. (1986) Interspecific interference competition: nest sites for blue and great tits. *Journal of Animal Ecology* 55, 331–350.

Mitchell, S. E., & Read, A. F. (2005) Poor maternal environment enhances offspring disease resistance in an invertebrate. *Proceedings of the Royal Society B* 272, 2601–2607.

Naef Daenzer, L., Naef Daenzer, B. & Nager, R. G. (2000) Prey selection and foraging performance of breeding great tits *Parus major* in relation to food availability.

Journal of Avian Biology 31, 206–214.

Palinauskas, V., Kosarev, V., Shapoval, A., Bensch, S. & Valkiūnas, G. (2007) Comparison of mitochondrial cytochrome b lineages and morphospecies of two avian malaria parasites of the subgenera Haemamoeba and Giovannolaia (Haemosporida: Plasmodiidae). *Zootaxa* 1626, 39–50.

R Development Core Team, 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Ricklefs, R., & Fallon, S. (2004) Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. *Systematic Biology* 53, 111-119.

Sadd, B. M., & Schmid-Hempel, P. (2009) A distinct infection cost associated with trans-generational priming of antibacterial immunity in bumble-bees. *Biology Letters* 5, 798–801.

Santiago-Alarcon, D., Valkiunas, G., Schaefer, H. M. (2012) Dipteran vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. *Biological Reviews* 87, 928-964.

Sehgal, R. N. M., Buermann, W., Harrigan, R. J., Bonneaud, C., Loiseau, C., Chasar, A., Sepil, I., Valkiūnas, G., Iezhova, T., Saatchi, S. & Smith, T. B. (2011) Spatially explicit predictions of blood parasites in a widely distributed African rainforest bird. *Proceedings of the Royal Society B* 278, 1025–1033.

Sepil I., Lachish, S., Hinks, A. E., & Sheldon, B. C. (2013) *Mhc* supertypes confer both qualitative and quantitative resistance to avian malaria infections in a wild population. *Proceedings of the Royal Society B* **280,** 20130134. doi:10.1098/rspb.2013.0134

Stauss, M. J., Burkhardt, J. F. & Tomiuk, J. (2005) Foraging flight distances as a

measure of parental effort in blue tits *Parus caeruleus* differ with environmental conditions. *Journal of Avian Biology* 36, 47–56.

Tschirren, B., Fitze, P. S. & Richner, H. (2007) Maternal modulation of natal dispersal in a passerine bird: an adaptive strategy to cope with parasitism? *The American Naturalist* 169, 87–93.

Valkiūnas, G. 2005. Avian malaria parasites and other haemosporidia. Boca Raton, CRC Press.

Waldenström, J., Bensch, S., Hasselquist, D. & Östman, Ö. (2004) A new nested polymerase chain reaction method very efficient in detecting *Plasmodium* and *Haemoproteus* infections from avian blood. *Parasitology* 90,191-194.

Westerdahl, H., Asghar, M., Hasselquist, D. & Bensch, S. (2012) Quantitative disease resistance: to better understand parasite-mediated selection on major histocompatibility complex. *Proceedings of the Royal Society B* 279, 577–584.

Wild, G., Gardner, A. & West, S. A. (2009) Adaptation and the evolution of parasite virulence in a connected world. *Nature* 459, 983–986.

Wilkin, T. A., Garant, D., Gosler, A. G. & Sheldon, B. C. (2006) Density effects on life-history traits in a wild population of the great tit *Parus major*: analyses of long-term data with GIS techniques. *Journal of Animal Ecology* 75, 604–615.

Wilkin, T. A., Perrins, C. M. & Sheldon, B. C. (2007) The use of GIS in estimating spatial variation in habitat quality: a case study of lay-date in the great tit *Parus major*. *Ibis* 149, 110–118.

Wolinska, J., & King, K. C. (2009) Environment can alter selection in host-parasite interactions. *Trends in Parasitology* 25, 236–244.

Wood, M. J., Cosgrove, C. L., Wilkin, T. A., Knowles, S. C. L., Day, K. P. & Sheldon, B. C. (2007) Within-population variation in prevalence and lineage distribution of

avian malaria in blue tits, *Cyanistes caeruleus*. *Molecular Ecology* 16, 3263–3273.

Supporting Information

The following Supporting Information is available for this article online: "Supporting Information.doc".

Tables and figure legends

Table 1: Relative ability of prevalence on natal and breeding territories to explain infection status among breeding blue tit recruits. All analyses controlled for natal year as a factor, and included 342 recruits from n=265 broods. Significant effects ($p<0.05$) are shown in bold.

		P. circumflexum			***P. relictum***		
Local prevalence measure	df	χ^2	p	Parameter estimate (SE)	χ^2	p	Parameter estimate (SE)
Natal site only	1	**14.64**	**0.0001**	**2.79 (0.735)**	0.03	0.8689	-0.18 (1.11)
Natal site (controlling for breeding site)	1	2.59	0.1075	1.33 (0.813)	0.05	0.8260	-0.25 (1.132)
Breeding site only	1	**33.70**	**<0.0001**	**4.18 (0.754)**	0.09	0.7610	0.30 (0.998)
Breeding site (controlling for natal site)	1	**21.65**	**<0.0001**	**3.67 (0.813)**	0.11	0.7361	0.34 (1.013)

Table 2: The effect of natal territory prevalence and postnatal dispersal distance on malaria infection probability. Terms in the minimal model are shown in bold, while statistics for removed terms are reported at the point that factor left the model. Both analyses include 442 recruits from 342 broods.

		P. circumflexum		*P. relictum*	
Predictor	**df**	χ^2	**p**	χ^2	**p**
Natal year	4	**8.93**	**0.0628**	**29.87**	**<0.0001**
Natal prevalence	1	-	-	0.34	0.5582
Dispersal distance	1	-	-	**6.18**	**0.0129**
Natal prevalence*Dispersal distance	1	**6.52**	**0.0107**	1.26	0.2614

Table 3: The relationship between parental and offspring malaria infection status among breeding blue tits. Parental infection status is from the year the offspring was born, and offspring status is in their first monitored breeding attempt. All analyses include brood as a random effect, and control for recruit age and natal year.

		P. circumflexum				*P. relictum*			
Parental variable	**Df**	***n***	χ^2	**p**	Parameter estimate (SE)	***n***	χ^2	**p**	Parameter estimate (SE)
Maternal status	1	305	2.84	0.092	0.69 (0.393)	305	1.51	0.220	-0.44 (0.366)
Paternal status	1	309	**7.14**	**0.008**	**0.92 (0.337)**	309	0.00	0.987	-0.01 (0.417)
N parents infected	1	266	**11.59**	**0.001**	**0.92 (0.268)**	266	0.39	0.533	-0.17 (0.271)

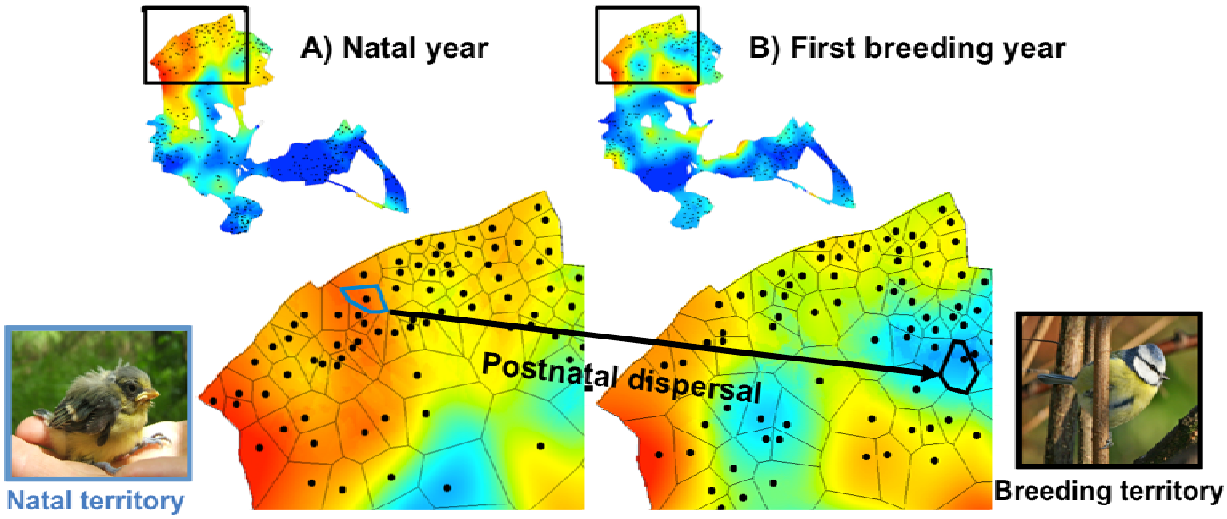

Fig. 1: Illustration of the analytical approach used to examine where and when infection most likely occurs for two sympatric avian malaria parasite species. For each recruit, two proxies of local infection risk were derived: the interpolated prevalence on their natal territory during their nestling phase (A), and interpolated prevalence on their breeding territory during their first breeding attempt (B). The relative ability of these two measures to predict recruit infection status was used to assess at which life-history stage infection occurs. The effect of postnatal dispersal distance on infection probability, and whether it altered the predictive power of natal site prevalence was also assessed. In the figure, dots represent nestboxes, territories are indicated by Thiessen polygons, and heat colours shows the interpolated prevalence derived from breeding adults in that year (excluding all recruits in the dataset), increasing from blue (low prevalence) to red (high prevalence). *Photo credit for adult blue tit: Joe Tobias*

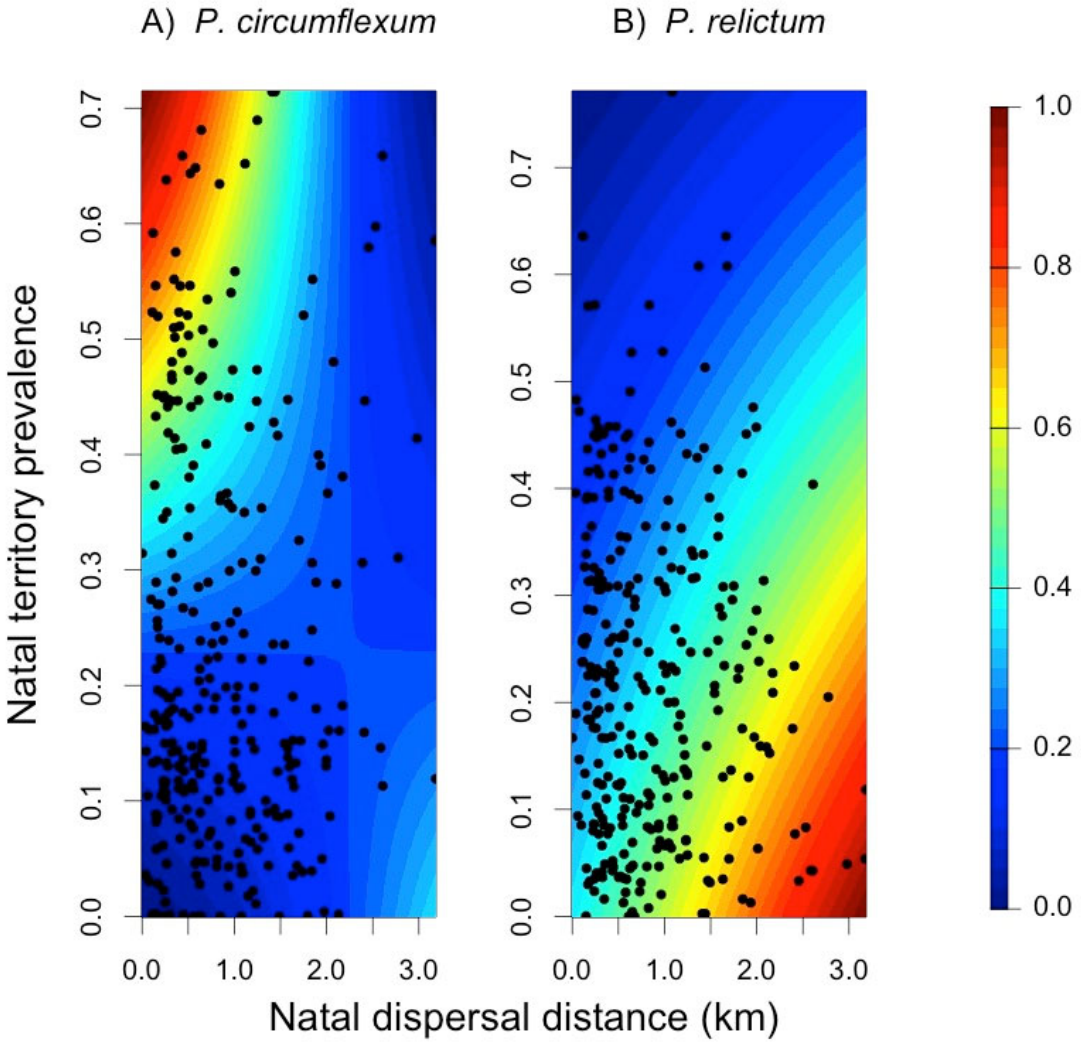

Fig. 2: The effect of natal territory prevalence and postnatal dispersal distance on infection of probability for A) *Plasmodium circumflexum* and B) *P. relictum*, among breeding blue tit recruits Colour indicates the probability of infection from blue (low probability) to red (high probability). Heat surfaces were generated using parameter estimates from a GLMM including the interaction term between local prevalence on the natal territory and postnatal dispersal distance. This interaction was significant for *P. circumflexum*, whereas only a main effect of dispersal distance was supported for *P. relictum* (see Table 3).

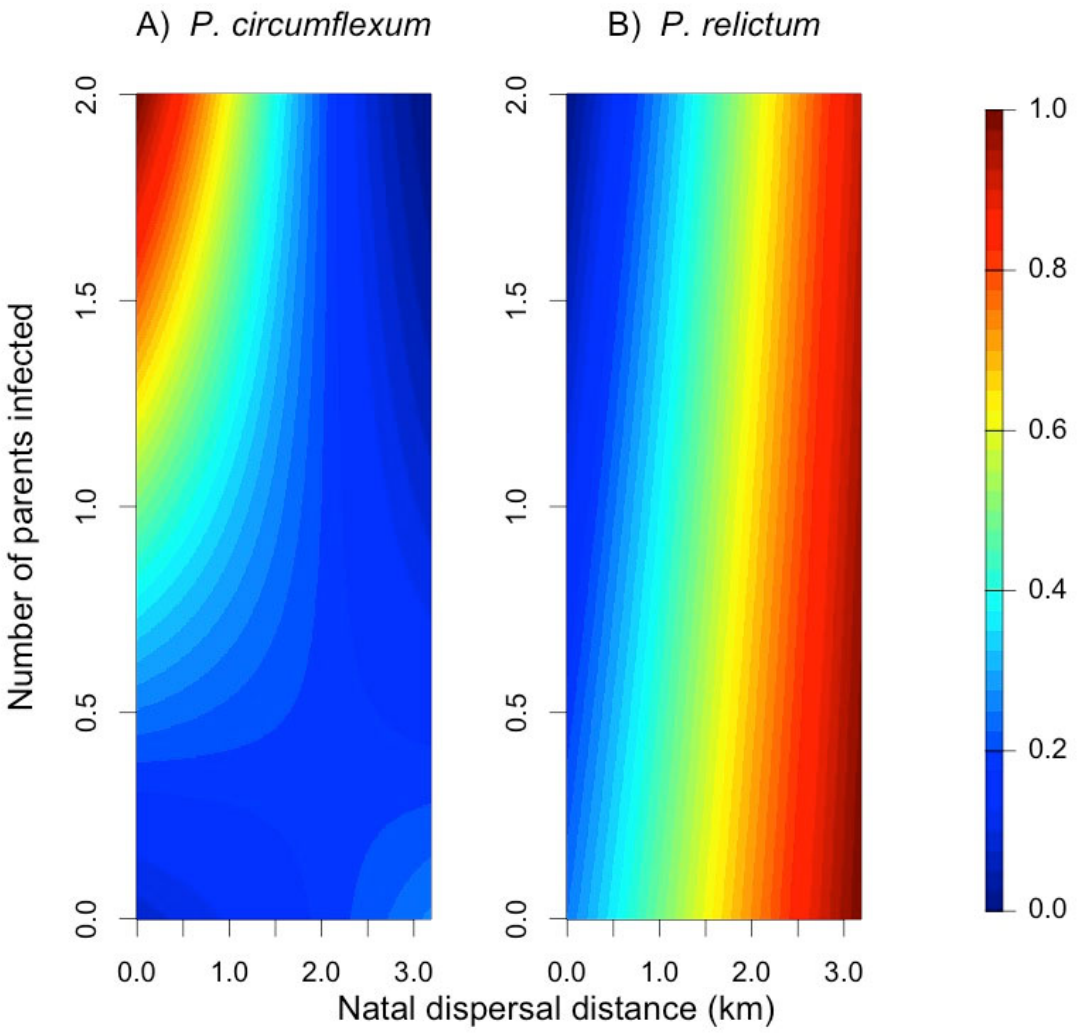

Fig. 3: The effect of parental infection and postnatal dispersal distance on infection of probability for A) *Plasmodium circumflexum* and B) *P. relictum*, among breeding blue tit recruits. Colour indicates the probability of infection from blue (low probability) to red (high probability). Heat surfaces were generated using predicted values from a GLMM including the interaction term between the number of parents infected and postnatal dispersal distance.